HEPATITIS C IN NEONATES

THESIS

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By

ASHRAF MAHMOUD ABDEL MONEM
M.B., B.Ch.
Faculty of Medicine – Ain Shams University

Supervisors

PROF. DR. KHALIL ABDEL HADY MOURAD
Professor of Pediatrics
Faculty of Medicine – Ain Shams University

DR. NANCY ABDEL AZIZ SOLIMAN
Assist. Professor of Pediatrics
Faculty of Medicine - Ain Shams University

BRIG. GEN. DR. MAHMOUD SHAKER IBRAHIM
Head of Laboratory Division
Military Medical Academy

Faculty of Medicine

Ain Shams University

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INTRODUCTION

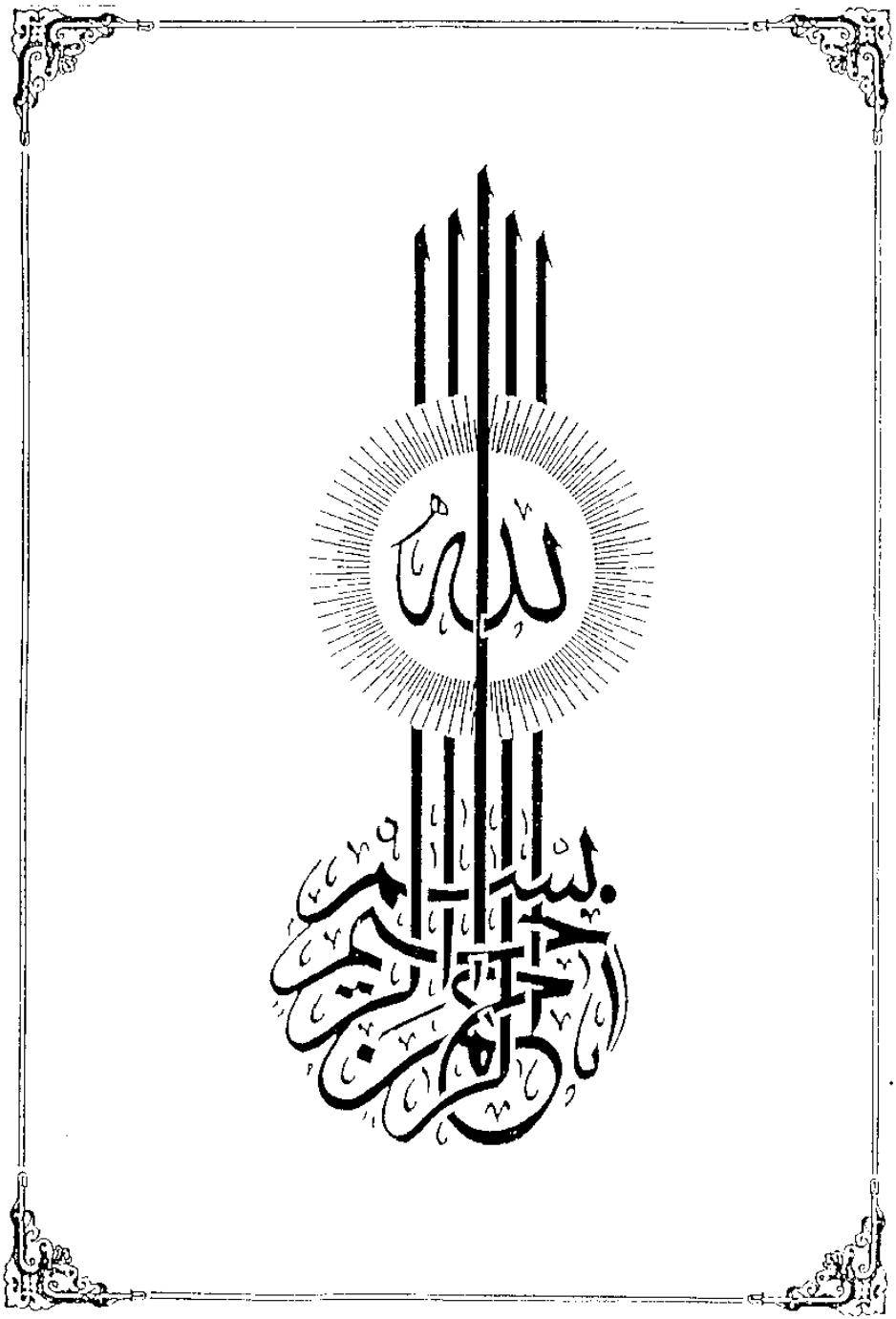
Hepatitis C virus (HCV) is an important transmissible disease, which has a global distribution. In developed countries, it is the most common form of post-transfusion hepatitis, and may account for more than 90% of the cases (*Arima et al.*, 1989; *Choo et al.*, 1989).

Isolation and characterization of the genome of HCV showed that it is an enveloped single strand RNA virus with a genome of approximately 10,000 nucleotides, a diameter of 50–60 nm, and most likely belongs to Flavi virus family (*Choo et al.*, 1989).

HCV infection has a short incubation period (15–50 days) and an ominous propensity to progress to chronic liver disease. Up to 50% of individuals with acute post-transfusion hepatitis due to HCV develop chronic hepatitis (*Huth et al.*, 1989).

In approximately 20 percent of patients, cirrhosis may develop insidiously within ten years. Hepatocellular carcinoma (HCC) associated with chronic hepatitis C is now recognized (*Muchmore et al.*, 1989).

HCV is mainly transmitted by parenteral route, sexual transmission and transmission by saliva are also reported (*Alter et al.*, 1989).



Risk factors for HCV infection seem to be similar to hepatitis B virus (HBV) infection, and some groups like haemophilics, intravenous drug abusers and patients on renal dialysis are more susceptible.

Perinatal transmission of HCV infection has been reported by some authors. *Tong et al.* (1987) reported that about 50% of infants born to women with acute HCV infection during the third trimester of pregnancy would have antibodies against HCV later in their lives.

Aim of the Work

The objectives our study are to identify:

1. Infection rate of HCV in the studied group of mothers.
2. Infection rate among their babies at delivery.
3. To correlate between the infection in both groups.

Review of Literature

MOLECULAR CHARACTERIZATION OF HCV

The aetiological agent responsible for most cases of post-transfusion non-A, non-B (NANB) hepatitis has been cloned and characterized (*Choo et al.*, 1989, 1991). This virus, now termed hepatitis C virus (HCV), is a positive strand RNA virus distantly related to pestivirus and flavivirus (*Millers and Purcel*, 1990; *Koonin*, 1991). Its genome consists of an approximately 332 nucleotides, 5` no-coding region (5` NCR), followed by a continuous single open reading frame encoding a polypeptide of around 3010 amino acids, and then a short 3` untranslated region contains between 27 and 55 nucleotides, depending on the source of the virus (*Kato et al.*, 1990; *Choo et al.*, 1991; *Takamizawa et al.*, 1991).

The 5` NCR represents the most highly conserved region among different viral isolates (*Kato et al.*, 1990; *Choo et al.*, 1991; *Han et al.*, 1991; *Okamoto et al.*, 1991; *Takamizawa et al.*, 1991).

By analogy with flavivirus, this polypeptide has been divided into a 5` structural region (5` NCR) consists of putative core and envelop proteins, and 3` region corresponding to non-structural (NS1 to NS5) proteins (*Kuo et al.*, 1989; *Muraiso et al.*, 1990; *Hosein et al.*, 1991, *Reyes and Baroudy*, 1991). (Fig. 1).

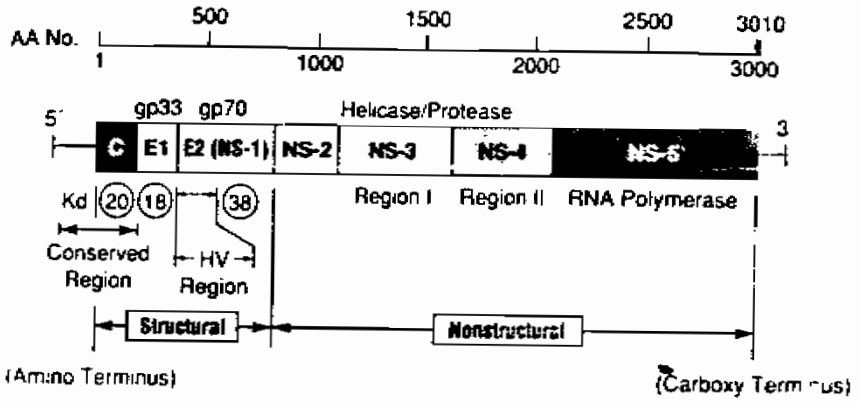


Fig. 1: Putative structure of the HCV genome. The genome consists of approximately 10,000 nucleotide bases coding for 3,000 amino acids. Structural genes are located at the 5' end of the genome and include the core or nucleocapsid *(C)* and the envelope *(E1, E2)* regions. Five non-structural regions *(NS-1 to NS-5)* extend to the 3' end of the genome. Highly conserved sequences are found in the 5' untranslated region and in the core. A hypervariable region is found in the 5' untranslated region and in the core. A hypervariable region is found in the E2 *(NS-1)* domain. First-generation anti-HCV assay utilize a protein *(c100-3)* in the NS-3 region. Second-generation assays will fuse an additional non-structural protein, c33c, to c-100-3 to form a new composite antigen designated c200. In addition, second-generation assays will detect antibody to a nucleocapsid antigen, c22-3. (Quoted from *Houghton et al.*, 1991).

Like its pestivirus and flavivirus relatives, HCV does not appear to produce DNA replication intermediates, and integrated forms of the viral genome in the host genome have not been detected (*Choo et al.*, 1989).

Recently, evidence of subgenomic HCV-RNA species has been obtained using polymerase chain reaction (PCR) analysis (*Han et al.*, 1991).

Trials of Viral Isolation

Watanabe et al. (1992) defined ultrastructural feature of circulating virus particles in the blood donor with NANB hepatitis. It was found that the feature of visualized virus particles showed pleomorphism in size, and appearance, the majority of them was 50–80 nm spherical particles with a 24–45 nm inner core, and 7–10 nm surface spike-like projections. In others, large spherical particles of more than 100 nm in diameter, and rod-like forms with 7–10 nm surface projections were also observed. The fractions containing these particles were positive for HCV-RNA. They concluded that these evidences strongly indicate that the pleomorphic virus particles are probably HCV, but it will be further required to confirm the specific reactivity of the particles to an antibody to the structural protein of HCV.

HCV Diversity

There is a considerable heterogenicity in the HCV, particularly in the viral envelop region (*Houghton et al.*, 1991).

E1, E_2-NS (envelop region 1 & 2), represents variable and hypervariable regions of amino acid sequence (the least conserved parts of HCV genome). These variable and hypervariable regions (in E_1, E_2-NS) observed in different HCV genomes suggest an ability of this virus to evade the immune surveillance by rapid mutation as has been observed in other viruses (*Payne et al.*, 1987).

Houghton et al. (1991) classified HCV into three genotypes: HCV-I (HCV prototype group), HCV-II (HCV-j group) and HCV-III (HCV-K_2 group). The 2nd and 3rd group represent Japanese isolates.

Recently, *Chan et al.* (1992) conducted a more sophisticated study involving HCV diversity, using different PCR primers, they reported that phylogenetic analysis revealed the existence of 3 distinct groups of sequences and that the variability in the nucleotide sequences of 5` NCR inside each group is less than 3%, while ranging from 9% between groups I and II and between groups I and II to 13% between groups II and III.

A more marked variability has been observed in the regions NS3 and NS5, unlike the 5` NCR where there are only three distinct groups, each of the coding regions shows prominent differentiation into separate clusters, as the case in HCV group I which is separated into two separate clusters with different geographical distribution (HCV-1 in USA/Europe and HCV-J1, HCV-BK in Japan). It was concluded that the major types of HCV (3 basic groups) could conceivably represent distinct serotypes, each capable of human infection irrespective of the immune response mounted against other HCV types.

The finding of mixed infection of different types of HCV in multiply exposed individuals, such as haemophilics, has been reported (*Enomoto et al.,* 1990; *Chan et al.,* 1991; *Nakao et al.,* 1991; *Pozzato et al.,* 1991).

Chan et al. (1992) reported also that the existence of different HCV types open up the possibility that the distinct disease syndromes associated with HCV infection may reflect underlying differences in the pathogenicity of the different types of the virus. There is some evidence that infection with HCV type III leads to more severe disease than type I and is less susceptible to interferon treatment

There is also strong association between HCV type III and intravenous drug abuse (*Pozzato et al.*, 1991).

The degree of sequence variability found between HCV types would be expected to affect profoundly the antigenicity of many of the putative proteins of HCV, hence donors infected with different HCV types show distinct differences in the pattern of reactivity to a range of structural and non-structural proteins in two commercial immunoblot assay for HCV antibody (*Chan et al.*, 1991). In particular, no reactivity with C-100 antigen and infrequent reactivity with C-33 antigen were observed in patients infected with HCV types II and III presumably reflecting the high degree of sequence variability in the NS_3 and NS_4 regions of the genome. Reactivity, however, was always found with the core protein antigen, which is consistent with the degree of sequence conservation in this region. This then provides at least one explanation for the observation that blood donors screening with the original C100-based immunoassay reduced the incidence but did not entirely prevent post-transfusional NANB hepatitis (*Esteban et al.*, 1990).

MODE OF TRANSMISSION OF HCV

HCV is mainly transmitted by parenteral route, about 90% of post-transfusion hepatitis is caused by HCV (*Choo et al.*, 1989).

Haematogenous Routes of Infection

Persons commonly considered at high risk of acquiring HCV include blood and blood product recipients, parenteral drug users, health care workers with occupational exposure to blood, and haemodialysis patients. These groups, however, only account for half of the hepatitis C cases reported in USA (*Alter et al.*, 1990).

Richard et al. (1991) reported that 91% of cases of post-transfusion hepatitis are caused by HCV, and nearly all cases of NANB post-transfusion hepatitis are caused by HCV, so, screening with a second generation assay improves the rate of detection of HCV both in patients with post-transfusion hepatitis, and blood donors.

Moreover, *Tremolada et al.* (1991) shows the cumulative rate of anti-HCV seroconversion in relation to the time of onset of hepatitis in patients with post-transfusion hepatitis which reached 90% by the end of one year from the onset of hepatitis.

Kuo et al. (1989) and *Alter et al.* (1989) found that 93% of acute post-transfusion hepatitis in patients receiving frequent blood transfusions are due to HCV.

The chance of contracting HCV infection is directly related to the number of units of blood or blood products transfused (*Schramm et al.*, 1989).

In haemophilics, the prevalence of anti-HCV correlated with the amount of blood products received showed that 75% of patients who were treated with less than 100 units of plasma per kilogram body weight per year had antibodies against HCV compared to 90% in patients who were treated with 100–300 units, and approached 100% in patients treated with more than 300 units plasma per kg body weight per year.

Makris et al. (1990), in a study on 154 patients with haemophilia, reported that the prevalence of anti-HCV was associated with exposure to clotting factor concentrates. 76 of 129 (59%) who had received factor VIII or IX had an anti-HCV, 42 of 55 (76%) who required over 10,000 units of concentrate annually had anti-HCV, compared with 34 of 74 (46%) who required less, and 0 of 25 patients who had never received concentrates. They found that 5 of 23 (22%) were only treated with heated concentrates had anti-HCV compared with 71 of 106 (67%)

patients who received unmodified products. This observation is consistent with reports that heat treatment of the concentrates reduces, but does not abolish the risk of NANB hepatitis (*Colombo et al.*, 1985; *Perston et al.*, 1985; *Kemoff et al.*, 1987).

HC and Haemodialysis

A preliminary survey in the dialysis units in Madrid City Hospital in Spain revealed that among patients in HBV free units 20% had antibodies against HCV and persistent alanine transaminase (ALT) activity. The risk of HCV infection was associated significantly with recent blood transfusion in patients undergoing haemodialysis, while for staff, the risk factor that correlated with HCV infection was a recent needle prick (*Esteban et al.*, 1989; *Zeldis et al.*, 1990).

Occupational Transmission

Robert et al., (1991) reported that health care workers have an occupational risk of infection with HCV. In New York City, dentists were tested for seropositivity for HCV. 8 (1.75%) of 456 dentists were positive for anti-HCV compared with 1 (0.14%) of 723 controls. They concluded that dentists are at a higher risk for HCV infection and that all health care workers should regard patients as potentially infected with communicable blood borne agent.

Non-Haematogenous Routes of Transmission of HCV

Percutaneous spread of HCV infection only accounts for half of the cases of hepatitis C reported in USA (*Alter et al.*, 1990).

Bortolotti et al. (1991) reported that 50% of community acquired NANB hepatitis are HCV positive and that these patients have no risk factors such as blood transfusion or drug abuse and their mode of infection is uncertain.

Vertical Transmission

Kamitsukasa et al. (1989) suggested that transmission of HCV by means other than blood transfusion is common. They detected HCV antibody in families of 2 patients including a son of infected mother born 4 years after the mother had been transfused.

Wejstal and Norkans (1989) reported that 1 of 11 children born to 8 women with chronic HCV infection during pregnancy had active anti-HCV production and concomitant liver disease. All children tested before 6 months of age were positive for anti-HCV at most up to 7 months of age and then became negative except one who had active anti-HCV production.